PLASMA LH CONCENTRATIONS FOR PREPUBERTAL, POSTPUBERTAL, ANESTROUS AND CYCLIC EWES OF VARYING FECUNDITY[1,2]

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SUMMARY

Luteinizing hormone (LH) was measured in plasma samples collected at 15-min intervals for 2 hr from 40 prepubertal Finn, Finn-cross (1/2), Rambouillet and Hampshire ewes at 9, 13 and 17 weeks of age and on Day 5 of the second estrous cycle after puberty. Blood samples were collected at 20-min intervals from 40 yearling and 20 mature ewes of the same breeds during anestrus and on Day 5 of the estrous cycle. Ovulation rates were determined by laparotomy on Day 5 of the estrous cycle.

The plasma LH pattern for the prepubertal females was characterized by pulsatile LH releases which decreased ($P<.01$) in magnitude with advancing age. Overall mean plasma LH concentration for the yearling ewes was higher ($P<.01$) than for the mature ewes (2.39 *vs* 1.38 ng/ml). Plasma LH concentrations for the yearling ewes were higher ($P<.05$) at mid-anestrus than late anestrus. Ovulation rate was significantly higher for the Finn ewes (lambs, $P<.05$; yearlings, $P<.01$), but was not correlated to plasma LH concentration within or among breeds.

(Key Words: LH, Prepubertal, Postpubertal, Fecundity, Sheep.)

INTRODUCTION

Merino ewes selected for a high *vs* low incidence of multiple births for several generations (Turner, 1969) were used by Bindon *et al.* (1971), who reported that ovarian sensitivity to exogenous gonadotropins was greater in the high- than in the low-ovulation group. Lamond and Emmens (1959) reported that hypophysectomy decreased the response of the mouse uterus to gonadotropins and suggested that the potency of exogenous gonadotropin is dependent on the endogenous gonadotropin concentration. The higher incidence of multiple births and increased ovarian sensitivity to exogenous gonadotropins observed by Bindon *et al.* (1971) may have resulted from a correlated response for higher endogenous gonadotropin concentrations in Merino ewes selected for multiple births. The purpose of this study was to determine whether the difference in ovulation rates among Finnsheep (Finn), Finn-Rambouillet cross (1/2), Hampshire and Rambouillet ewes is related to LH concentrations in the peripheral plasma of prepubertal and postpubertal ewes.

MATERIALS AND METHODS

Blood samples were collected from 40 prepubertal Finn (10), Finn-cross (10), Rambouillet (10), and Hampshire (10) ewes at 9, 13 and 17 weeks of age and on Day 5 of the second estrous cycle after puberty. Puberty was defined as the first standing behavorial estrus. Blood samples were obtained from 40 yearling and 20 mature ewes of the same breeds at mid-anestrus (July 12, 1973), late anestrus (August 9, 1973) and on Day 5 of the second estrous cycle (October or November, 1973). Vasectomized rams equipped with markers were used to monitor estrus continuously.

Jugular vein blood samples (10 ml) were collected at 15-min intervals from the lambs and at 20-min intervals from the mature ewes for a period of 2 hours. Plasma LH concentrations were determined by use of the double antibody radioimmunoassay for ovine LH described by Niswender *et al.* (1969) with modification as described by Lewis *et al.* (1974); NIH-

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LH-S 18 was used as the ovine standard. The antibody DJB 3-12/11 bound in excess of 90% of the LH-^{125}I at a 1:300 dilution and 45 to 50% at the assay dilution of 1:35,000. Two plasma pools were assayed in nine separate assays and had a mean LH concentration of .9 and 3.3 ng/ml with interassay coefficient of variations of 10.1 and 5.5%, respectively. Three concentrations (.2, 1 and 5 ng) of NIH-LH-S18 were quantitatively recovered from .2 ml of plasma. Unknown plasma samples were assayed at .1 and .2 ml and the coefficient of variation was less than 15%, indicating a parallel response of the standards and unknowns.

Ewe lambs and yearlings were laparotomized on Day 5 of the estrous cycle after the blood collections to determine ovulation rates. Ovulation rates for Finn-cross (1/2), Hampshire and Rambouillet mature ewes at this laboratory have been reported previously (Laster and Glimp, 1974).

Expression of plasma LH concentration as means or by distribution frequency of the LH concentrations resulted in similar ranking among animals within breeds and among breeds; therefore, results are expressed as mean LH concentrations by day, age and breed (table 1). Data were analyzed by a split-plot analysis of variance and Duncan's multiple-range test (Steel and Torrie, 1960). Within and among breed comparisons of plasma LH concentration and ovulation rate were made by use of regression and analyses.

RESULTS AND DISCUSSION

The sequential plasma samples from the prepubertal ewes contained pulsatile LH releases as previously reported by Liefer *et al.* (1972) for 10- to 44-day-old ewe lambs. Disappearance of the LH peaks occurred at a rate similar to the 28-min half-life obtained by Foster *et al.* (1972). The single LH surge observed per lamb during the 2-hr collection period suggested that the interval between LH peaks may be greater than 2 hr and account for the absence of a LH peak in a few animals. The magnitude of the LH peaks decreased with advancing age between 9 and 17 weeks of age, accounting for the effect of age ($P<.01$) on plasma LH concentration (table 1). The percentage of ewe lambs having LH concentrations $\geq$4.0 ng/ml plasma was 65% at 9 weeks, 60% at 13 weeks and 54% at 17 weeks. Likewise, Bindon and Turner (1974) reported that the incidence of plasma LH peaks >10 ng/ml is higher for lambs at 30 days of age than 100 days. The daily administration of 15 to 20 μg exogenous estradiol to both intact and ovariectomized postnatal female lambs will suppress the LH fluctuations (Liefer *et al.*, 1972), suggesting the LH surges result from either insufficient production of ovarian steroids to suppress gonadotropin secretion or the responsiveness of the hypothalamo-hypophyseal system to ovarian steroid feedback increases during the postnatal development period. In turn, the pulsatile release of LH may aid in the stimulation of postnatal follicular development and steroidogenesis.

Ovulation rate varies both within and among breeds of sheep. A higher ($P<.05$) ovulation rate was observed for the Finnsheep than for the Finn-cross (1/2), Rambouillet or Hampshire postpubertal ewe lambs. There is some evidence that plasma LH concentration for prepubertal ewes positively reflects the fecundity of the breed or genetic population. Bindon and Turner (1974) compared both mean plasma LH concentrations and number of LH concentrations exceeding 3, 10 and 20 ng/ml at 30 and 100 days of age for lambs with a genetic difference in fecundity and found LH to be higher in the high fecundity flock at 30 days but not at 100 days of age. Data from the present study does not indicate a within or among breed relationship between fecundity and either mean plasma LH concentration or the distribution of the LH concentrations for lambs 56 days of age or older. Age of the lambs may be critical and account for the absence of a relationship between LH and ovulation rates as the lambs were 26 days older than animals used in the study by Bindon and Turner (1974).

Because plasma LH concentrations were higher ($P<.01$) for the yearling than for the mature ewes (2.39 *vs* 1.38 ng/ml), data for the two age groups were analyzed separately. Short-term LH fluctuations were observed in the plasma samples collected from the yearling ewes on July 12, 1973, but not for the mature ewes which contributed to the age difference in plasma LH concentrations. The reduction in magnitude of these LH peaks at the subsequent sampling periods resulted in a time effect ($P<.05$). Episodic releases of LH have been previously reported for anovulatory (Butler *et al.*, 1972) and anestrous (Yuthasastrokosal *et al.*, 1975) ewes. The age and day effects on plasma LH concentration for the anestrous

TABLE 1. MEAN PLASMA LH CONCENTRATIONS AND OVULATION RATES FOR PREPUBERTAL AND POSTPUBERTAL ANESTROUS AND CYCLIC EWES

Group	Finnsheep	Finn-cross (1/2)	Rambouillet	Hampshire	Mean[b]
			(ng/ml)		
Ewe lambs					
No. of animals	10	10	10	10	
9 weeks	4.7 ± 1.6	3.3 ± .6	4.0 ± .9	5.3 ± .9	4.3 ± .5[c]
13 weeks	2.8 ± .5	2.4 ± .3	3.0 ± .6	3.5 ± .7	2.9 ± .3[d]
17 weeks	1.8 ± .4	1.9 ± .5	2.2 ± .5	3.9 ± .9	2.4 ± .3[de]
Day 5 of cycle	1.5 ± .2	1.1 ± .2	2.2 ± .7	1.6 ± .3	1.6 ± .2[e]
Mean LH, ng/ml	2.7 ± .5	2.2 ± .2	2.9 ± .4	3.6 ± .4	2.8 ± .2
Ovulation rate[a]	2.2 ± .4	1.3 ± .2	1.3 ± .3	1.2 ± .1	1.5 ± .1
Yearling ewes					
No. of animals	10	10	10	10	
7-12-73	2.0 ± .4	3.4 ± .5	4.5 ± .7	4.3 ± .6	3.6 ± .3[f]
8-9-73	1.4 ± .2	3.5 ± 1.8	1.4 ± .4	1.7 ± .4	2.0 ± .5[g]
Day 5 of cycle	1.4 ± .2	1.4 ± .2	1.8 ± .3	1.9 ± .3	1.6 ± .1[g]
Mean LH, ng/ml	1.6 ± .2	2.8 ± .6	2.5 ± .4	2.7 ± .3	2.4 ± .5
Ovulation rate[a]	3.2 ± .5	1.8 ± .3	1.5 ± .2	1.7 ± .2	2.1 ± .1
Mature ewes					
No. of animals	5	5	5	5	
7-12-73	1.0 ± .3	.9 ± .1	.8 ± .1	1.1 ± .4	1.0 ± .1[h]
8-9-73	1.3 ± .3	1.1 ± .1	1.2 ± .3	2.1 ± .6	1.4 ± .2[hi]
Day 5 of cycle	1.8 ± .3	2.1 ± .8	1.5 ± .2	1.7 ± .3	1.8 ± .2[i]
Mean LH, ng/ml	1.4 ± .2	1.4 ± .3	1.2 ± .1	1.6 ± .3	1.4 ± .1

[a]Ovulation rate for Finnsheep is significantly different (ewe lambs, $P<.05$; yearling ewes, $P<.01$) from underscored means.

[b]Means within the ewe lamb, yearling ewe or mature ewe groups with the same superscripts are not significantly different ($P<.05$).

ewes may reflect differences in ovarian status since both estrogen and progesterone suppress peripheral LH concentrations in ovariectomized ewes (Hauger *et al.*, 1975). The reduction in magnitude of the LH peaks could also reflect a seasonal rhythm.

Within and among breed comparisons of plasma LH concentration and ovulation rate for the anestrous and cyclic yearling and mature ewes also indicated that plasma LH was not affected by, or correlated to, fecundity of the animal or breed. The absence of a relationship between plasma LH and ovulation rate on Day 5 of the estrous cycle is consistent with the observation of Land *et al.* (1973). Their comparison of plasma LH concentrations among breeds of sheep indicated that plasma LH was related to fecundity of the breed only on Days 1 and 8 of the ovine estrous cycle and in their study some of the breed differences in plasma LH concentrations were very small compared to the differences in fecundity. Land (*personal communication*) later indicated that plasma LH concentration for cyclic ewes was not related to fecundity.

Available data indicate that the use of plasma LH concentration as a tool to select ewes for fecundity offers only limited potential. The relationship between peripheral plasma LH and ovulation rate is highly variable and age dependent (Bindon and Turner, 1974; Thimonier *et al.*, 1972), which may explain the absence of a relationship between LH and fecundity in the present study.

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